UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: May 07, 2014

SUBJECT: **FLUENSULFONE:** Report of the Cancer Assessment Review Committee

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The Cancer Assessment Review Committee (CARC) met on March 12, 2014 to evaluate the cancer classification of fluensulfone in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

Fluensulfone

PC CODE: 050410

March 12, 2014
Submitted by: Jaime D'Agostino, PhD

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

Fluensulfone is a new fluoroalkenyl nematicide proposed for pre-plant control of root knot nematodes in cucurbits and fruiting vegetables by occupational handlers. Guideline studies were conducted in rats and mice to evaluate the carcinogenic potential of fluensulfone. Additionally, all available data on toxicity, metabolism, mutagenicity, Structure Activity Relationships (SAR), and Mode of Action (MOA) were considered in this evaluation. On March 12, 2014, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs evaluated the carcinogenic potential of fluensulfone.

Male and female Wistar rats received oral administration of fluensulfone (96.7% pure) in the diet at 0, 30, 200 or 1200 ppm (0/0, 1.4/1.7, 9.6/11.6, and 57.7/69.3 mg/kg bw/day in males/females) for 104 weeks (MRID 48574765). No treatment-related tumors were observed. The CARC considered the highest dose tested in both sexes to be adequate and not excessive to assess the carcinogenic potential of fluensulfone. This determination was based on the results of the 90-day study which was used for dose selection and the findings in the 104-week carcinogenicity study which included decreased body weights, clinical chemistry changes, and the presence of non-neoplastic lesions in the lungs and esophagus.

Male and female CD-1 mice received oral administration of fluensulfone (96.7-98.7% pure) in the diet at 0, 30, 200, or 1200 ppm (0/0, 4.2/6.4, 27.6/39.0, and 152.3/188.4 mg/kg bw/day in males/females) for 78-weeks (MRID 48574766). Alveolar/bronchiolar adenomas were seen in the lungs of female mice at both the mid and high doses. The increases in adenomas and combined adenomas/carcinomas reached statistical significance and were outside of the historical control range for adenomas at both dose levels. In addition, the presence of this tumor was corroborated by the occurrence of bronchiolization (hyperplasia) in the lungs of both sexes at both the mid and high doses. Based on these considerations, the CARC concluded that the alveolar/bronchiolar adenomas of the lung in female mice are treatment-related. No treatment-related tumor findings were observed in male mice. The CARC considered the doses tested in both sexes to be adequate, but not excessive, to assess the carcinogenic potential of fluensulfone. This determination was based on the results of the 90-day study which was used for dose selection and the findings in the 78- week carcinogenicity study which included decreased body weight, clinical chemistry changes, non-neoplastic lesions in the lungs and neoplastic lesions (females only) in mice in the main study.

Based on the available guideline genetic toxicology data, there is no mutagenic concern for fluensulfone. Additional genetic toxicology studies were also performed with three major fluensulfone metabolites, thiazole sulfonic acid, butene sulfonic acid, and methyl sulfone. Based on the results of the genetic toxicology studies, it was also concluded that there is no mutagenic concern for the fluensulfone metabolites tested.

An analysis of structure activity relationships for fluensulfone provided limited additional information. A single structural analog, tetrafluoroethylene, was identified. Tetrafluoroethylene resulted in a different toxicological and carcinogenic profile than what was observed for

fluensulfone and did not provide any relevant information or support regarding the carcinogenic potential of fluensulfone.

A postulated Mode of Action (MOA) for the formation of lung tumors in mice was submitted by the registrant. The MOA involved the following proposed key events: (1) extensive metabolism of fluensulfone by mouse lungs, predominantly by cyp 2f2 that produces reactive metabolites; (2) induced Clara cell proliferation; (3) increased proliferation leading to bronchiolar/alveolar hyperplasia; and (4) hyperplasia progression to bronchiolar/alveolar adenomas and carcinomas. The CARC evaluated the available data to support the proposed MOA and concluded that the evidence was insufficient to support a non-genotoxic MOA for lung tumors. This decision was based primarily on a lack of sufficient data to: (1) establish that metabolism in the lung is a required key event; (2) demonstrate that Clara cell proliferation occurred at or below the tumorigenic doses; and (3) demonstrate that the cell proliferation was Clara cell specific.

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005): **Fluensulfone is classified as "Suggestive Evidence of Carcinogenic Potential"** based on the following considerations:

(i) Treatment-related alveolar/bronchiolar adenomas of the lung in female mice at doses that were considered adequate to assess carcinogenicity;

(ii) No treatment-related tumors were observed in either sex of rats at doses that were considered adequate to assess carcinogenicity;

(iii) There is no mutagenicity concern from the *in vivo* or *in vitro* genetic toxicity assays; and

(iv) There is insufficient data to support the proposed non-genotoxic MOA.

The CARC recommended using a non-linear approach (i.e., reference dose (RfD)) that will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to fluensulfone.

I. INTRODUCTION

On March 12, 2014, the CARC of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of fluensulfone.

II. BACKGROUND INFORMATION

Fluensulfone (Figure 1) is a pre-plant nematicide of the fluoroalkenyl class for the control of root knot nematodes. Fluensulfone is a new active ingredient (ai) undergoing joint review with Canada and Australia. Fluensulfone is proposed for occupational use only to control root knot nematodes in cucurbits and fruiting vegetables. The pesticidal mode of action is undefined; however, it appears to affect multiple physiological processes resulting in death of the nematode.

The mammalian mode of action has not been identified. The majority of dietary exposure is expected to be to two metabolites of fluensulfone, butene sulfonic acid (BSA) and thiazole sulfonic acid (TSA) (Figure 1), as these were the primary residues observed in plant metabolism and field trial studies. In addition, methyl sulfone (MS) (Figure 1) was found to be an environmental degradate of fluensulfone and a major metabolite in water. The Residues of Concern Knowledgebase Subcommittee (ROCKS) concluded that the parent, fluensulfone, should be the residue of concern for risk assessment of primary crops (see ROCKS memo, D412114) as oral acute toxicity data indicates that BSA and TSA are significantly less toxic than fluensulfone (LD_{50} of 671 mg/kg for fluensulfone vs. >2000 mg/kg for BSA and TSA). The ROCKS also recommended to include MS as a residue of concern for risk assessment in drinking water as the oral acute toxicity data for MS indicated that MS has similar toxicity to fluensulfone (LD_{50} between 300 and 2000 mg/kg for MS).

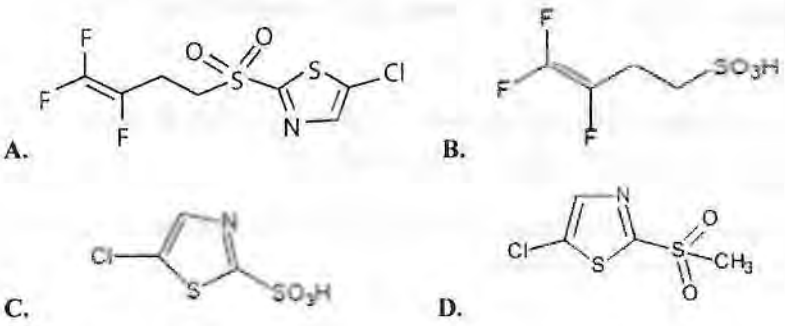

Figure 1. A. Fluensulfone B. Butene Sulfonic Acid (BSA) C. Thiazole Sulfonic Acid (TSA) D. Methyl Sulfone (MS)

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Carcinogenicity Study in Rats – Fluensulfone

Reference: Kaiser, S. (2011) MCW-2 TECH: 104-Weeks (Feeding) Combined Chronic Toxicity and Oncogenicity Study in Wistar Rats. Project Number: R/23353/OCR, B80188. Unpublished, MRID 48574765.

A. Experimental Design- Rat Study

In an oral combined chronic toxicity/carcinogenicity study in rats (MRID 48574765), 4 groups of HANRcc Wist (SPF) rats (allocation A, for carcinogenicity evaluation), 50 males and 50 females each, were treated for 104 weeks with 0, 30, 200 or 1200 ppm (0/0, 1.4/1.7, 9.6/11.6, and 57.7/69.3 mg/kg bw/day in males/females) of fluensulfone (MCW-2 tech., 96.7%, Batch No. NLL6692-3) in their diets. A satellite group (allocation B, for chronic toxicity evaluation) of 20 males and 20 females per group was similarly treated for 52 weeks at the same dose levels (0/0, 1.6/1.9, 11.0/13.1, and 66.3/75.2 mg/kg bw/day in males/females).

B. Discussion of Survival Data- Rat Study

Mortality was not affected by treatment with fluensulfone in males or females for animals allocated for the 52-week sacrifice (Tables 1 and 2).

Table 1. Fluensulfone – Wistar Rat Study (MRID 48574765)
Male Mortality Rates[+] for Animals Allocated to 104-week Sacrifice

Dose (ppm)	Weeks			
	1-35	36-71	72-105[f]	Total
0	1/50	0/49	10/49	11/50 (22)
30	0/50	1/50	7/49	8/50 (16)
200	0/50	4/50	11/46	15/50 (30)
1200	1/50	2/49	9/47	12/50 (24)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at weeks 105.
( ) Percent.
Note: Time intervals were selected for display purposes only.

Table 2. Fluensulfone – Wistar Rat Study (MRID 48574765)
Female Mortality Rates[+] for Animals Allocated to 104-week Sacrifice

Dose (ppm)	Weeks			
	1-35	36-71	72-105[f]	Total
0	0/50	0/50	14/50	14/50 (28)
30	0/50	1/50	13/49	14/50 (28)
200	0/50	2/50	15/48	17/50 (34)
1200	1/50	1/50	11/49	12/50 (24)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at weeks 105.
( ) Percent.
Note: Time intervals were selected for display purposes only.

C. Neoplastic Lesions- Rat Study

There were no treatment-related tumors reported. All neoplastic findings were within the range of normal background lesions for the sex and animal strain used in this study.

D. Non Neoplastic Lesions- Rat Study

After 104 weeks (terminal sacrifice), an increased number of focus/foci in the lungs were recorded in animals at 1200 ppm (statistically significant in males) (Table 3). Slight increases in incidences of foci and cysts in livers of females at 1200 ppm, and foci in the prostate of males at 200 and 1200 ppm were also noted. The lung focus/foci were mostly correlated with foci of alveolar macrophages and/or foci of chronic interstitial inflammation of the lung.

After 52 weeks, hyperkeratosis of the esophagus was recorded at a minimal severity grade in animals of both sexes at 1200 ppm (data not shown). After 104 weeks, an increased incidence of hyperkeratosis was recorded in both males and females at 200 and 1200 ppm (Table 3). Statistical significance was recognized in both males and females (Fisher's Exact $p<0.01$) at 1200 ppm only and there was a positive trend in both sexes by the Armitage trend test ($p<0.01$). A dose-related increase in severity was not observed.

There were no microscopic findings in the lungs after 52 weeks of treatment. After 104 weeks, there was an increase in incidence of chronic interstitial inflammation in the lungs recorded in both males and females at 1200 ppm and females at 200 ppm, which was only statistically significant in the high dose male group as compared to the control group by Fisher's Exact pairwise comparison ($p<0.01$) (Table 3). Statistical significance was recognized in both males and females by Armitage trend test ($p<0.01$). The chronic interstitial inflammation was characterized by focal/multifocal changes consisting of interstitial or intra-alveolar inflammatory cells associated with hypertrophied reactive type II pneumocytes. These lesions were associated with foamy intra-alveolar macrophages, which were slightly increased in mean severity grade when compared to the lungs of control male and female rats. The lesions were non-hyperplastic.

Table 3. Fluensulfone – Wistar Rat Study (MRID 48574765)
Non-Neoplastic Lesions for Animals Allocated to 104-week Sacrifice

Findings	0 ppm n = 48-50		30 ppm n = 48-50		200 ppm n = 48-50		1200 ppm n = 48-50	
	M	F	M	F	M	F	M	F
Lung: Focus/foci	3 [6%]	4 [8%]	5 [10%]	8 [16%]	5 [10%]	9 [18%]	15* [30%]	11 [22%]
Liver: Focus/foci	7 [14%]	0 [0%]	9 [18%]	2 [4%]	6 [12%]	3 [6%]	10 [20%]	4 [8%]
Cysts	0 [0%]	3 [6%]	1 [2%]	1 [2%]	1 [2%]	1 [2%]	1 [2%]	8 [16%]
Prostate: Focus/foci	1 [2%]		3 [6%]		5 [10%]		5 [10%]	
Esophagus: Hyperkeratosis	3 [6%] (1.0)	2 [4%] (1.5)	2 [4%] (1.0)	3 [6%] (1.0)	7 [14%] (1.0)	8 [16%] (1.3)	21* [42%] (1.1)	20* [42%] (1.4)

Table 3. Fluensulfone – Wistar Rat Study (MRID 48574765) Non-Neoplastic Lesions for Animals Allocated to 104-week Sacrifice								
Findings	0 ppm n = 48-50		30 ppm n = 48-50		200 ppm n = 48-50		1200 ppm n = 48-50	
	M	F	M	F	M	F	M	F
Lung: Chronic interstitial inflammation	2 [4%] (1.5)	3 [6%] (1.0)	4 [8%] (1.2)	1 [2%] (1.3)	2 [4%] (1.7)	7 [14%] (1.3)	13* [26%] (1.5)	9 [18%] (1.4)
Lungs: Alveolar macrophages	34 [64%] (1.1)	31 [63%] (1.0)	26 [52%] (1.2)	33 [66%] (1.3)	26 [52%] (1.7)	39 [80%] (1.3)	32 [64%] (1.5)	33 [66%] (1.4)

* Significant p <0.01 (Fisher's Exact).
[] Percent. () Severity.

E. Adequacy of Dosing for Assessment of Carcinogenicity

The doses selected for the carcinogenicity study were based on the results of the 90-day dietary toxicity study in rats (MRID 48574754). Fluensulfone was administered in the diet at 0, 60, 120, 500, and 2000 ppm (equivalent to average daily intakes of 0/0, 4.31/4.85, 8.26/11.68, 34.86/53.10, and 139.01/148.73 mg/kg/day in males/females, respectively). Increased severity of hyaline droplet accumulation in the kidney (5 incidences of both grade 2 and grade 3 at 500 ppm vs. 6 incidences at grade 1 and 4 incidences at grade 2 in the controls) and an increased incidence of stomach hyperplasia (4 vs. 0 in the controls) and mononuclear infiltrates in the pharynx (4 vs. 2 in the control) was observed at 500 ppm in males. The increased incidence of stomach hyperplasia was also observed in females at 500 ppm (4 vs. 0 in the controls). In addition, females had a 25% elevated serum cholesterol level at 500 ppm. At 2000 ppm, multiple other findings were observed including a 9-12% decrease in body weight in males and a 44% increase in reticulocyte count in females. No mortality was seen at 2000 ppm. Based on these results the doses of 0, 30, 200, or 1200 ppm were chosen for the 104-week study. The CARC determined that the doses selected were adequate and not excessive based on the findings in the 90-day subchronic study (described above) and the findings in the chronic/carcinogenicity study which included treatment-related changes in body weight hematology clinical chemistry parameters, and histopathology (lung and esophagus). There was no effect on survival in the carcinogenicity study.

2. Carcinogenicity Study in Mice – Fluensulfone

Reference: Kaiser, S. (2011) MCW-2 TECH: 78-Weeks Oncogenicity (Feeding) Study in CD-1 mice. Project Number: R/23354/OCR, B80190. Unpublished, 2616 p., MRID 48574766.

A. Experimental Design- Mouse Study

In an oral oncogenicity study (MRID 48574766), 4 groups of 50 male and 50 female CD-1 mice were treated for 78 weeks with 0, 30, 200 or 1200 ppm of fluensulfone (MCW-2 tech., 96.7-98.65%, Batch No. 36372130-291-PF1) in their feed. Additionally, 6 males and 6 females were

exposed to the same dietary concentrations and used for liver enzyme determination after 13 weeks of treatment. The average daily intakes of test material were 0, 4.2, 27.6 or 152.3 mg/kg bw/day for males and 0, 6.4, 39.0 or 188.4 mg/kg bw/day for females.

B. Discussion of Survival Data- Mouse Study

There were no statistically significant survival disparities among the dose groups with increasing doses of fluensulfone (Table 4).

Table 4. Fluensulfone – CD-1 Mouse Study (MRID 48574766)
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks			
	1-26	27-52	53-80[f]	Total
0	0/50	0/50	7/50	7/50 (14)
30	0/50	2/49[a]	10/47	12/49 (24)
200	0/50	2/50	12/48	14/50 (28)
1200	0/50	1/50	13/49	14/50 (28)

[+]Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[a]One accidental death at week 49, dose 30 ppm.
[f]Final sacrifice at weeks 78-80.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Neoplastic lesions- Mouse Study

Female mice had a significant trend for alveolar/bronchiolar combined adenomas and/or carcinomas at $p < 0.05$. There were significant pair-wise comparisons of the 200 ppm dose group with the controls for alveolar/bronchiolar adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. There were also significant pair-wise comparisons of the 1200 ppm dose group with the controls for alveolar/bronchiolar adenomas and combined adenomas and/or carcinomas, both at $p < 0.05$. The statistical analyses of the tumors in the female mice were based upon the Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Table 5). No statistically significant tumor findings were observed in male mice.

Table 5. Fluensulfone – CD-1 Mouse Study (MRID 48574766) Female Alveolar/Bronchiolar Tumor Rates[+] and Fischer's Exact Test and Exact Trend Test Results					
	Dose (ppm)				HC
Tumor Type	0	30	200	1200	
Adenomas (%)	2/50 (4)	4/47 (9)	14/48 (29)	9[a]/49 (18)	9/265 (3%) (0-6%)
p =	0.09379	0.30959	0.00068**	0.02346*	
Carcinomas (%)	2/50 (4)	1/49 (2)	1/49 (2)	4[b]/50 (8)	9/226 (3%) (0-10%)
p =	0.09142	0.87504	0.87504	0.33887	
Combined (%)	3[c]/50 (6)	5/49 (10)	15/49 (31)	12[c]/50 (24)	Not Provided
p =	0.03467*	0.34609	0.00140**	0.01130*	

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52 (adenomas) or week 49 (carcinomas and combined).

[a]First adenoma observed at week 55, dose 1200 ppm.
[b]First carcinoma observed at week 49, dose 1200 ppm.
[c]One animal in each of the control and 1200 ppm dose groups had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

D. Non Neoplastic lesions- Mouse Study

An increase in the incidence and severity of bronchiolization (hyperplasia) was seen in the lung of males and females at 200 and 1200 ppm (Table 6). Morphologically this finding mainly consisted of a change from flattened epithelium to cuboidal epithelium, often with hypertrophy of the non-ciliated epithelium (Clara cells) lining the terminal bronchioles. In the highest dose group, this change extended to the adjacent alveolar walls. Transmission electron microscopy was used to confirm that the hypertrophy of the epithelium of the terminal bronchioles was affecting mostly the non-ciliated Clara cells. The registrant stated that the Clara cells increased in number giving rise to a pseudo-stratified epithelium extending occasionally to the respiratory bronchioles and alveolar ducts.

Table 6. Incidence data of non-neoplastic lesions for male and female CD-1 mice in the 78-week oncogenicity study of fluensulfone.				
	Dose (ppm)			
Males	0	30	200	1200
Bronchiolization (hyperplasia)	1/50	0/50	24*/50	31*/50
Mean severity grade	1.0	-	1.3	1.6
Females				
Bronchiolization (hyperplasia)	5/50	7/50	43*/50	48*/50
Mean severity grade	1.0	1.0	1.8	2.6

* $p < 0.01$ using Fisher's Exact Test (one-sided)

E. Adequacy of Dosing for Assessment of Carcinogenicity

The doses selected for the carcinogenicity study were based off of a 90-day dietary study in mice (MRID 48574753). Fluensulfone was administered in the diet at 0, 60, 300, and 1500 ppm (equivalent to average daily intakes of 0/0, 11/18, 51/68, and 228/252 mg/kg/day in males/females, respectively). Changes in hematology (a 13% increased hematocrit and a 34% increase in white blood cell count in males) and a 45-54% increase in bilirubin in both sexes was observed following treatment with 300 ppm fluensulfone. At 1500 ppm, additional effects were observed including a 10% decrease in male and female body weight on the final day of the study, a 32-36% increase in reticulocyte count in both sexes, a 23-38% decrease in platelets in both sexes, a 55-107% increase in the activity of aspirate aminotransferase, alanine aminotransferase, and alkaline phosphatase in both sexes, and findings of hepatocellular hypertrophy, bile duct hyperplasia, oval cell proliferation, degeneration, and pigment deposits in the livers of both sexes. Mortalities were observed at all dose levels; however, there was no dose response and the mortalities were reported to be related to blood sampling at study termination, except for one female at 1500 ppm who died on day 7. Based on these results, the doses of 0, 30, 200, or 1200 ppm were chosen for the carcinogenicity study. The CARC determined that the doses selected were adequate and not excessive based on the findings in the 90-day subchronic study and the findings in the 78-week study including treatment related changes in body weight in both sexes, hematology, clinical chemistry, and non-neoplastic and neoplastic lesions in the lung. There was no effect on survival in the carcinogenicity study

IV. TOXICOLOGY

1. Metabolism

In a rat metabolism study (MRID 48574742), [^{14}C]Fluensulfone ([thiazole-^{14}C]MCW-2, 98.7-99.3%, and [butene-^{14}C]MCW-2, 96.8-98.8%,) was administered to male and female Wistar rats (4 animals/dose/sex) by gavage as a single dose at levels of 5 or 500 mg/kg. The recovery of radioactivity for all dose groups was 91.3-97.3% of the administered dose (AD). Urine was the major route of excretion for both radiolabel positions and was the only source of metabolites ≥5% of the AD. For the low-dose thiazole radiolabel, 84-86% AD was excreted in urine, 8-11% AD in feces, and 2% AD remained in the carcass and GI tract. A similar excretion profile was observed for the high-dose thiazole radiolabel, and both the high and low-dose butene labels. The major urinary metabolite for the thiazole radiolabel was a thiazole mercapture (38-40% AD and 49-54% AD, low- and high-dose, respectively) followed by thiazole glucuronides (24-27% AD and 20-22% AD, low- and high-dose, respectively), and thiazole sulfonic acid (3-5% AD and 1-3% AD, low- and high-dose, respectively). The major urinary metabolite for the butene radiolabel was a butene sulfinic acid (32-35% AD and 53-57% AD, low- and high-dose, respectively) followed by butene sulfonic acid (4-5% AD and 4% AD, low- and high-dose, respectively). Unmetabolized fluensulfone was either not detected as a significant residue, or at very low levels (0.1% AD in feces detected after multiple oral doses), in urine and feces with both radiolabels. No significant sex differences were observed.

In a rat metabolism study (MRID 48574744), [^{14}C]Fluensulfone ([thiazole-^{14}C]MCW-2, 98.5%) was administered to male and female Wistar rats (4 animals/dose/sex) by gavage as a single dose at levels of 5 mg/kg after the rats were given 14 repeated daily oral doses with unlabeled fluensulfone at 5 mg/kg/day. The recovery of radioactivity was 94-99% AD. Radiolabel was primarily found in the urine (71-83% AD), with lesser amounts in the feces (9-11% AD) and cage wash (6-16% AD). One day after dosing the highest tissue levels were in the GI tract (4.5-4.7% AD). The four major metabolites identified were thiazole sulfonic acid (2-3% AD), glucuronide I (20-23% AD), glucuronide II (6-8% AD), and thiazole mercapturate (39-45% AD). No significant sex differences were observed. The results from this study were similar to those observed following the single dose metabolism study above.

In a rat metabolism study (MRID 48574741), [^{14}C]Fluensulfone ([thiazole-^{14}C]MCW-2, 100%, and [butene-^{14}C]MCW-2, 96.5-97%,) was administered to male and female Wistar rats (8 animals/dose/sex) by gavage as a single dose at levels of 5 or 500 mg/kg to determine pharmacokinetic parameters for fluensulfone. Fluensulfone was rapidly absorbed with T_{max} values of 2-8 hours for each radiolabel at the low dose. The results were generally similar between males and females. The amount of radiolabel associated with whole blood increased relative to plasma with time and this effect was more pronounced with the thiazole label. It was determined using *in vitro* experiments that fluensulfone reacts with the free thiol moiety of globin to form a covalent linkage to the thiazole group displacing the butene sulfinic acid.

In a rat metabolism study (MRID 48574743), [^{14}C]Fluensulfone ([thiazole-^{14}C]MCW-2, 99-99.2%, and [butene-^{14}C]MCW-2, 96.6-96.7%,) was administered to male and female Wistar rats (3 animals/dose/sex) by gavage as a single dose at levels of 5 or 500 mg/kg to determine tissue distribution of fluensulfone. For both radiolabels and doses, the GI tract, liver, and kidney had the highest radioactive levels at C_{max}. The thiazole label was slowly eliminated from red blood cells and bone marrow consistent with the PK results. Similar results were observed between males and females.

2. Mutagenicity

Fluensulfone has been tested in a comprehensive battery of genetic toxicology studies (Table 7). The results indicate that fluensulfone is not mutagenic or clastogenic. While there was evidence of a weak mutagenic response (less than 2-fold over control) with *Salmonella typhimurium* strain TA100 in the absence of metabolic activation in a bacterial reverse mutation assay(MRID 48575015), it was not confirmed in two other bacterial reverse mutation assays. Based on the results of the genetic toxicology studies, it was concluded that there is no mutagenic concern for fluensulfone.

Three major metabolites of fluensulfone (thiazole sulfonic acid, butene sulfonic acid, and methyl sulfone) were also tested in a comprehensive battery of genetic toxicology studies (Table 8). All three metabolites were negative in all tests except for one weakly positive result (~2-fold over control) for methyl sulfone with *Salmonella typhimurium* TA100 in the absence of metabolic activation. There is little concern for the positive result as it was abolished by the presence of S9

and was not observed in other tester strains specific for base pair changes. Based on the results of the genetic toxicology studies, it was concluded that there is no mutagenic concern for the fluensulfone metabolites tested.

Table 7. Genetic Toxicology results for fluensulfone		
2002-Bacterial reverse mutation assay 48574759/48575015	Test 1: 0, 16, 50, 158, 500, 1581, or 5000 µg/plate (*Salmonella typhimurium*) in the presence and absence of metabolic activation (± S9). Test 2: 0, 1000, 2000, 3000, 4000, 5000, or 6000 µg/plate (*Salmonella typhimurium*) in the presence and absence of metabolic activation (± S9). Test 3: 0, 375, 750, 1300, 2600, or 5200 µg/plate (*Salmonella typhimurium*) in the absence of metabolic activation (-S9). Acceptable/Guideline	Negative with strains TA1535, TA1537, TA98, and TA102. Very weak response (<2-fold) with strain TA 100 at 5000 (test 1) and 4000, 5000, and 6000 (test 2) µg/plate in the absence of S9. Negative in test 3.
2008-Bacterial reverse mutation assay 48574760	Test 1: 0, 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Test 2: 0, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Acceptable/Guideline	Negative
2011-Bacterial reverse mutation assay 48574761	Test 1: 0, 10, 32, 100, 316, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Test 2: 0, 3, 10, 32, 100, 316, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Acceptable/Guideline	Negative
2003-*In vitro* mammalian cell gene mutation assay 48574763	Chinese Hamster Lung cells-V79 Without activation: 0, 24, 32, 40, 48, 56, 64, 72 µg/ml With activation: 0, 20, 30, 40, 50, 60, 70, 80 µg/ml Acceptable/Guideline	Negative Cytotoxicity was observed at 80 µg/ml and above with and without S9.
2010-*In vitro* mammalian cell chromosome aberration test 48574762	Human peripheral lymphocytes 0, 62.5, 125, 250, 500, 1000 µg/mL with and without metabolic activation Acceptable/Guideline	Negative Cytotoxicity was observed at 1000 µg/ml without metabolic activation (24-hour exposure) and 2500 µg/ml with metabolic activation (4-hour

Table 7. Genetic Toxicology results for fluensulfone		
		exposure).
2003-mammalian erythrocyte micronucleus test in mice 48574764	mg/kg = 0, 75, 150, 300, 2 intraperitoneal injections separated by 24 hours 300 mg/kg/day was selected as the maximum tolerable dose for males in a pilot test. Acceptable/Guideline	Negative Signs of toxicity (apathy, roughened fur, loss of weight, spasm, difficulty breathing, slitted eyes and closed eyes) were observed starting at 75 mg/kg/day. No toxicity to bone marrow was observed.

Table 8. Genetic Toxicology results for fluensulfone metabolites		
2010-Bacterial reverse mutation assay Thiazole sulfonic acid 48574784	Test 1: 0, 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Test 2: 0, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Acceptable/Guideline	Negative
2010-Bacterial reverse mutation assay Methyl sulfone 48574788	Test 1: 0, 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Test 2: 0, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Test 3: 0, 100, 333, 1000, 2500, 3750, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Acceptable/Guideline	Negative with strains TA 98, 1535, 1537, and WP2 uvrA. Very weak positive response (~2-fold) with strain TA 100 at 5000 (test 1), 2500 and 5000 (test 2), and 5000 (test 3) µg/plate in the absence of S9.
2010-Bacterial reverse mutation assay Butene sulfonic acid 48574793	Test 1: 0, 3, 10, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Test 2: 0, 33, 100, 333, 1000, 2500, or 5000 µg/plate (*Salmonella typhimurium-Escherichia coli*) in the presence and absence of metabolic activation (± S9). Acceptable/Guideline	Negative
2011-*In vitro* mammalian cell gene mutation assay Methyl sulfone 48574789	Chinese Hamster Lung cells-V79 Without activation: test1 = 0, 3.8, 7.5, 15, 30, 40, 50, 60 µg/ml, test 2 = 0, 5.5, 10.9, 21.9, 43.8, 87.5, 131.3, 175 µg/ml With activation: test 1 = 0, 37.5, 75, 150, 300, 600, 800, 1000 µg/ml, test 2 = 0, 87.5, 175, 350, 700, 800, 900, 1000 µg/ml	Negative Cytotoxicity was observed at 31.3 µg/ml without metabolic activation and 1000 µg/ml with metabolic activation (4-hour exposure).

Table 8. Genetic Toxicology results for fluensulfone metabolites		
	Acceptable/Guideline	Cytotoxicity was also observed at 62.5-1000 µg/ml in the absence of metabolic activation (24-hour exposure).
2010-*In vitro* mammalian cell chromosome aberration test Thiazole sulfonic acid 48574785	Chinese Hamster Lung cells-V79 Test 1: 0, 9.3, 18.5, 37, 74.1, 148.1, 296.3, 592.5, 1185, 2370 µg/mL with and without metabolic activation Test 2: 0, 9.3, 18.5, 37, 74.1, 148.1, 296.3, 592.5, 1185, 2370 µg/mL without metabolic activation and 148.1, 296.3, 592.5, 1185, 2370 µg/mL with metabolic activation Acceptable/Guideline	Negative No cytotoxicity was observed following treatment up to the highest concentration.
2010-*In vitro* mammalian cell chromosome aberration test Butene sulfonic acid 48574794	Chinese Hamster Lung cells-V79 Test 1: 0, 8.3, 16.6, 33.3, 66.6, 133.2, 266.4, 532.8, 1065.5, 2131 µg/mL with and without metabolic activation Test 2: 0, 8.3, 16.6, 33.3, 66.6, 133.2, 266.4, 532.8, 1065.5, 2131 µg/mL without metabolic activation and 133.2, 266.4, 532.8, 1065.5, 2131 µg/mL with metabolic activation Acceptable/Guideline	Negative No cytotoxicity was observed following treatment up to the highest concentration.
2011-mammalian erythrocyte micronucleus test in rat Thiazole sulfonic acid 48574786	mg/kg = 0, 500, 1000, 2000, single gavage dose in sterile water Acceptable/Guideline	Negative Reduced spontaneous activity at 4 hours post dosing was observed in some animals and ruffled fur was observed in all animals at 24 hours post dosing at 2000 mg/kg. No toxicity to bone marrow was observed.
2011-mammalian erythrocyte micronucleus test in rat Methyl sulfone 48574791	mg/kg = 0, 125, 250, 500, single gavage dose in corn oil Acceptable/Guideline	Negative Reduced spontaneous position and abdominal position was observed up to 4 hours post dosing and ruffled fur up to 24 hours post dosing in all animals treated with 500 mg/kg. A slight cytotoxic effect on the bone marrow was observed in 4 out

Table 8. Genetic Toxicology results for fluensulfone metabolites		
		of 7 animals at 500 mg/kg at the 48 hour sacrifice.
2010-mammalian erythrocyte micronucleus test Butene sulfonic acid 48574795	mg/kg = 0, 500, 1000, 2000, single gavage dose in sterile water Acceptable/Guideline	Negative Ruffled fur was observed up to 24 hours post dosing at 2000 mg/kg and up to 6 hours post dosing at 1000 mg/kg. No toxicity to bone marrow was observed.
2011-unscheduled DNA synthesis in rats Methyl sulfone 48574790	mg/kg = 0, 250, 500, single gavage dose in corn oil Acceptable/Guideline	Negative Reductions in spontaneous activity and ruffled fur were noted in animals treated with both 250 and 500 mg/kg. No effect on hepatocyte viability was observed.

3. Structure-Activity Relationship

Fluensulfone is a novel fluoroalkenyl nematicide. It is the first of this class of nematicides and, therefore, there are limited structural analogs for comparison. The available literature was screened for relevant information on structural analogs of fluensulfone. Tetrafluoroethylene was identified as a potential analog based on its structure (Table 9); however, it appears to result in a different toxicological (primarily targets the kidney in both rats and mice) and carcinogenic (liver tumors in mice and rats and kidney tumors in rats) profile than seen for fluensulfone (primarily targets the hematopoietic system in multiple species and causes lung tumors in mice). A brief discussion of the mutagenicity and carcinogenicity study results from NTP are provided for completeness.

Table 9. Structural analog of fluensulfone		
Chemical Name	**CAS #**	**Structure**
Tetrafluoroethylene	116-14-3	

Tetrafluoroethylene did not cause gene mutations in *Salmonella typhimurium* with or without mammalian metabolic activation. It also did not cause gene mutations in Chinese hamster ovary cells or micronucleus formation in peripheral-blood erythrocytes in mice exposed *in vivo*. **Tetrafluoroethylene is classified as a Group 2B carcinogen (possibly carcinogenic to**

humans) by IARC (IARC, vol 30). There is clear evidence of carcinogenic activity in animals based on increased incidence of renal tubule neoplasms and hepatocellular neoplasms in male F344/N rats, liver hemangiosarcomas, hepatocellular neoplasms, renal tubule neoplasms, and mononuclear cell leukemia in female F344/N rats, and liver hemangiomas, liver hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcoma in male and female B6C3F$_1$ mice (NTP 1997).

4. Subchronic and Chronic Toxicity

Fluensulfone targets multiple organs/systems following administration to rats, mice, and dogs in subchronic and chronic studies. The hematopoietic system appears to be the major target as effects were observed in all three species in both subchronic and chronic studies. In addition, effects on the hematopoietic system were observed at the lowest observed adverse effect level (LOAEL) in all studies except the 90-day rat study. Other organs affected include the kidney in subchronic and chronic rat studies, the lung in chronic rat and mice studies, and the liver in subchronic studies of all three species. The effects on the kidney observed in rat were consistent with accumulation of alpha-2-microglobin which is not considered relevant to humans (USEPA 1991). The following is a brief description of the subchronic and chronic studies.

A. Subchronic Toxicity Studies

i. Mice

In a 4-week dietary study (MRID 48575024), groups of 5 male and 5 female Crl:CD1 mice received fluensulfone (BYI 01921, MCW-2 technical, 97%) in their diet at 0, 100, 500 and 2000 ppm (equivalent to 0/0, 30/41, 101/155, and 375/353 mg/kg bw/day in males/females, respectively).

Decreased body weight (6-13%) was observed in males at 2000 ppm but not in females at any dose. A 13% decrease in platelets was observed at 500 ppm in males. Multiple red blood cell parameters were affected at 2000 ppm in both males and females including decreased platelets (13-20%), increased erythrocyte count (10%), increased hemoglobin (6-10%), increased hematocrit (7-11%), and increased reticulocytes (18-26%).

Increased relative liver weights (15%) in females at 500 ppm and decreased absolute kidney weights (20%) at 2000 ppm in males were observed. Multiple histopathological findings in the liver were observed at 2000 ppm in both sexes. These included observations of cytoplasmic alterations, single cell necrosis, and mitotic figures in the periportal region and bile duct hyperplasia in the portal region.

The no observed adverse effect level (NOAEL) of the study was considered to be 100 ppm for both sexes (30 and 41 mg/kg bw/day in male and female mice, respectively). The LOAEL for both sexes was 500 ppm (101 and 155 mg/kg bw/day in male and female mice, respectively), based on decreased platelets in males; based on the marginal change at this dose, it is considered

a threshold effect level.

In a 90-day dietary toxicity study (MRID 48574753), groups of 12 male and 12 female Crl:CD1 (ICR)BR mice received diets containing 0, 60, 300 and 1500 ppm (equivalent to 0/0, 11/18, 51/68 and 228/252 mg/kg bw/day in males/females, respectively) of fluensulfone (BYI 01921 technical, 97.0-97.8%, Batch No. NLL6692-7-5) for a period of 13 weeks.

Decreased body weight (7-10% in males and 10% in females) was observed in both males and females at 1500 ppm. Multiple hematological effects were observed at 300 ppm and above in males and females. These included increased hemoglobin (9%), hematocrit (15%), and white blood cells (49%) in males and decreased platelets (23-38%) in both sexes. Clinical chemistry revealed increases in aspartate aminotransferase (56-62%), alkaline phosphatase (55%), and alanine aminotransferase (85-107%) in both sexes at 1500 ppm and increased bilirubin (45-77%) in both sexes at 300 ppm.

Increased relative liver weights (10-18%) were observed in both sexes at 1500 ppm. Increased absolute liver weights (10%) were only observed in females at 1500 ppm. Increased liver weights were accompanied by liver histopathology consisting of hepatocellular hypertrophy, bile duct hyperplasia, oval cell proliferation, hepatocellular degeneration, and pigment deposits.

The NOAEL of the study was 60 ppm (equivalent to 11 mg/kg bw/day for males and 18 mg/kg bw/day for females) based on increased bilirubin in both sexes and hematology effects in the males at the LOAEL of 300 ppm (51 mg/kg bw/day for males and 68 mg/kg bw/day for females).

ii. Rats

In a 28-day oral toxicity study (MRID 48575023), groups of 5 male and 5 female Wistar rats received fluensulfone (BYI 01921, MCW-2 technical, 98.4-98.9%, Lot No. NLL6692-3) in their diet at 0, 125, 500 and 2000 ppm (0/0, 10.4/12.2, 42.5/37.2, 152.0/165.9 mg/kg bw/day in males/females) for approximately four weeks.

Decreased body weight was observed in males at 500 ppm (6-8%) and 2000 ppm (8-9%) and females (8-11%) at 2000 ppm. Increased leukocytes (27%) and lymphocytes (29%) were observed in females at 500 ppm but not in males. Changes in clinical chemistry parameters included increased cholesterol (17%) in males at 500 ppm and in females at 2000 ppm (14%). In addition, decreased triglycerides (25%) were observed in males at 500 ppm.

Relative liver (14%) and relative kidney (21%) weights were increased in male mice at 2000 ppm. Histopathology revealed changes consistent with alpha-2-microglobulin accumulation in the kidney, including basophilic tubules degeneration of proximal tubules, and hyaline droplets at 500 ppm in males.

The NOAEL of the study is 125 ppm (equivalent to 10.4 and 12.2 mg/kg bw/day in male and female rats, respectively). The LOAEL in this study is considered to be 500 ppm (equivalent to

42.5 and 37.2 mg/kg bw/day in male and female rats, respectively), based on reduced body weights in males, increased white blood cells in females, increased cholesterol in males, and renal pathology in males.

In a 90-day dietary toxicity study (MRID 48574754), groups of 10 male and 10 female Wistar rats (Hsd Cpb:WU) received diets containing 0, 60, 120, 500 or 2000 ppm of fluensulfone (BYI 01921, MCW-2 technical, 97%, Batch No. NLL6692-7-5) for a period of 3 months. The mean daily intakes in order of ascending concentration were 0, 4.31, 8.26, 34.86 or 139.01 mg/kg bw/day in males and 0, 4.85, 11.68, 53.10 or 148.73 mg/kg bw/day in females of the main groups.

Decreased body weights were observed in males (9-12%) and females (3-9%) at 2000 ppm. Increased reticulocytes (44%) were observed in females at 2000 ppm but not males. Clinical chemistry parameters affected included increased cholesterol (25%) in females at 500 ppm and increased triglycerides (73%) at 500 ppm.

Increased absolute and relative liver (7 and 17%) and kidney (9 and 21%) weights were observed at 2000 ppm in males. In contrast, only an increase in relative liver (9%) and kidney (14%) weights were observed in females at 2000 ppm. Histopathology revealed effects consistent with alpha-2-microglobulin accumulation in male rats at 500 ppm. Additional histopathological findings included hepatocellular hypertrophy in both sexes at 2000 ppm, basal cell hyperplasia in the forestomach of both sexes at 500 ppm, and mononuclear infiltrates in the pharynx of males at 500 ppm and females at 2000 ppm. Following necropsy, an increased level of fluoride was found in the bones and teeth of both sexes at all dose levels. However, there were no accompanying histopathological changes in teeth or bone.

As only adaptive effects on the liver were observed at 120 ppm, equivalent to 8.23 mg/kg bw/day for males and 11.68 mg/kg bw/day for females, 120 ppm is considered to be the study NOAEL. Based on the numerous effects see at 500 ppm in the diet (34.86 and 53.10 mg/kg bw/day in males and females, respectively) including in the kidneys, observations in the pharynx and forestomach, increased cholesterol in females, slightly decreased body weight in males, 500 ppm is considered the study LOAEL.

B. Relevant Chronic Toxicity Studies

i. Mice

In an oral oncogenicity study (MRID 48574766), 4 groups of 50 male and 50 female CD-1 mice (allocation A) were treated for 78 weeks with 0, 30, 200 or 1200 ppm of fluensulfone (MCW-2 tech., 96.7-98.65%, Batch No. 36372130-291-PF1) in their feed.

Decreases in body weight were observed in both males (8-20% over the course of the study) and females (12% at termination). Changes in hematological parameters were only observed in females and consisted of a decrease in white blood cells (26%) at 200 ppm. Effects on clinical

chemistry parameters included increased sorbital dehydrogenase (102-134%) in males and females at 200 ppm, and increased aspirate aminotransferase (34%) and alanine aminotransferase (85%) in females at 200 ppm.

Multiple organ weights were increased and decreased, but based on the lack of histopathological findings these were considered of no toxicological significance. Non-neoplastic lesions consisted of a statistical significant increase in the incidence of bronchiolization (i.e. a change from flattened epithelium to cuboidal epithelium) in the lung of males and females treated at 200 and 1200 ppm (from control to high dose respectively, 1, 0, 24 and 31 in males and 5, 7, 43 and 48 in females; n = 50 all groups). Transmission electron microscopy (TEM) analysis revealed hypertrophy of the epithelium of the terminal bronchioles affecting mostly the non-ciliated Clara cells as well as the directly surrounding ciliated cells.

The LOAEL was established at 200 ppm (27.35 and 38.96 mg/kg bw/day in males and females, respectively), based on decreased body weight/weight gain in males and an increased incidence of lung bronchiolization in males and females. The NOAEL was established at 30 ppm (4.2 and 6.4 mg/kg bw/day in males and females, respectively).

ii. Rats

In an oral combined chronic toxicity/oncogenicity study in rats (MRID 48574765), 4 groups of HANRcc Wist(SPF) rats (allocation A, oncogenicity animals), 50 males and 50 females each, were treated for 104 weeks with 0, 30, 200 or 1200 ppm (0/0, 1.4/1.7, 9.6/11.6, and 57.7/69.3 mg/kg bw/day in males/females) of fluensulfone (MCW-2 tech., 96.7%, Batch No. NLL6692-3) in their diets.

Body weight decreases (11%) were only seen in male rats at 1200 ppm at the end of the study. After 52 weeks, a number of changes in hematology and biochemistry parameters were noted, mainly in animals treated with 1200 ppm. Dose-dependent decreases in neutrophil (19-38%) and white blood cell counts (10-24%) were observed in all female dose groups. Clinical chemistry findings in the low and mid dose groups included changes in alanine aminotransferase, sodium, chloride, calcium, phosphorus, total protein and globulin, in males and/or females. Clinical chemistry and organ weight data revealed some effects on the liver, limited to the 1200 ppm group. In the absence of correlating histopathological findings these changes were considered to be non-adverse metabolic adaptations. Other organ weight changes noted in high dose groups included increased relative kidney weights in males (17%) and females (11%), and decreased adrenal weights in males (30-52%). After 104 weeks a few changes in hematology parameters, mainly in red blood cell parameters, were noted in animals treated with 1200 ppm.

At necropsy after 104 weeks, an increased number of foci in the lungs were recorded in the treated animals, compared to controls, attaining statistical significance only in males at 1200 ppm. Enlarged livers were recorded in 4 females (versus one in the control group), as well as cysts in the livers of 8 females (versus 2 in the control group) and foci in the liver of 4 females (versus 0 in the control group) treated at 1200 ppm. In males, a slight increase in foci in the

prostate was noted in mid and high dose groups (5 in each treatment group versus one in the control group). No correlative microscopic observations were noted in the prostate. Microscopically, an increased incidence of hyperkeratosis in the esophagus was recorded in animals of both sexes treated at 200 and 1200 ppm. Since the severity grade was minimal in most cases, the hyperkeratosis was considered to be of no toxicological significance, and possibly from a slight irritating effect of fluensulfone. The incidence of chronic interstitial inflammation in the lungs was increased in males and females treated at 1200 ppm and in females treated at 200 ppm. This lesion was characterized by focal/multifocal changes consisting of interstitial or intra-alveolar inflammatory cells associated with hypertrophied reactive type II pneumocytes, associated with the presence of foamy intra-alveolar macrophages, which was also increased in mean severity grade. There were no treatment-related hyperplastic changes or tumors recorded. At 104 weeks, slight increases were observed in chronic nephropathy, tubular basophilia and mononuclear foci in the kidneys and mononuclear infiltrates in the pharynx of females treated at 1200 ppm as compared to controls.

After 52 weeks of treatment, fluoride measurements revealed a marked dose-dependent increase, several fold above controls, of the fluoride content in the ashes from bones and teeth at 200 and 1200 ppm. Only very slight increases in fluoride content in bones (up to 28% above controls) were noted in 30 ppm animals. No increase of the fluoride content in the ashes from teeth was recorded in animals treated with 30 ppm. After 104 weeks of treatment, the fluoride content in the ashes from bones and teeth of all treated animals was markedly increased. Changes in fluoride levels were not considered adverse as there were no associated structural signs of dental fluorosis (discoloration of the teeth) or skeletal fluorosis (mobility problems, changes in external appearance of bones, changes in bone histopathology) at any dose.

The NOAEL was established at 200 ppm (9.6/11.6 mg/kg/day, m/f) based on decreased body weight, hematology and clinical chemistry findings, effects on liver, kidney and adrenal weight, and histopathological changes in the lungs and esophagus.

Fluensulfone showed no carcinogenic potential in rats. Dosing was considered adequate in both sexes based on the findings of decreased body weight and weight gain, slight hematological effects and microscopic findings in the lung and esophagus.

5. Mode of Action Studies

The Registrant submitted an analysis of Mode of Action (MOA) data in the International Programme on Chemical Safety framework (MRID 48575041), which has also been published in the literature (Strupp et al., 2012). The postulated MOA involves activation of fluensulfone to reactive metabolites by mouse lung Clara cells, primarily by mouse-specific cytochrome P450 2f2. The reactive metabolites result in increased cell proliferation that leads to lung hyperplasia and neoplasia.

A. Postulated Key Events for the MOA

The sequence of events in the proposed MOA for induction of lung tumors by fluensulfone is as follows:

- Extensive metabolism of fluensulfone by mouse lung, predominantly by Cyp 2f2, that produces reactive metabolites. **(Key Event #1)**
- Clara cells undergo increased proliferation. **(Key Event #2)**
- Increased proliferation leads to bronchiole-alveolar hyperplasia. **(Key Event #3)**
- Progression of bronchiolo-alveolar hyperplasia to adenomas and carcinomas. **(Key Event #4)**

B. Studies Available for the MOA Analysis

Two mechanistic studies were conducted to further describe the effects of fluensulfone on the lung and support the postulated MOA. The results of these studies are summarized in the registrants document (MRID 48575041). These include:

1. Comparative biotransformation study of fluensulfone between mice and human lung microsomes (MRID 48574768): The metabolic activity of human and mice lung microsomes was determined by incubation of lung microsomes with fluensulfone in the presence of an NADH-regenerating system. The remaining (unmetabolized) fluensulfone was determined by liquid chromatography-tandem mass spectrometry at different time points. Two concentrations of fluensulfone were tested (2 and 20 μM); however, the highest concentration was found to be too high (only a small percentage was metabolized) and the results obtained were considered only as confirmatory. The metabolism of mouse and human microsomes was further investigated in the presence and absence of specific inhibitors of CYP 2E1/cyp 2e1 (4-methyl pyrazole) and cyp 2f2 (5-phenyl-1-pentyne). Lung microsomes were prepared from 12 female and 12 male untreated mice, and their activity towards fluensulfone was compared with that of lung microsomes pooled from at least 10 different human non-smokers.

2. A mechanistic study to analyze local cell proliferation in lung (MRID 48574767): Groups of 10 CD-1 female mice were treated via the diet with 0 ppm or 1200 ppm fluensulfone for 3 days or 7 days. An additional 10 females each were administered 1305 ppm isoniazid (positive control) for 3 or 7 days. Approximately 14 and 2 hours prior to necropsy, all animals received an intraperitoneal injection of bromodeoxyuridine (BrdU) for determination of cell proliferation. Lungs, brain, and a piece of the small intestine were collected from all animals at necropsy. The weights of the lungs and brain were recorded. Lungs and intestine were processed for the determination of BrdU positive cells and analyzed histopathologically.

C. Data Supporting the Key Events in the MOA for Fluensulfone

1. Key event #1: Extensive metabolism by mouse lung, predominantly by cyp 2f2.

In an in vitro study (MRID 48574768), metabolic activity was determined by incubation of lung microsomes with fluensulfone in the presence of a nicotinamide adenine dinucleotide-regenerating system, and analytical determination of remaining (unmetabolized) fluensulfone by LC-MS/MS at different time points. Mouse lung microsomes prepared from male and female CD-1 mice were active toward fluensulfone resulting in turnover of approximately 90% of 2 µM fluensulfone to unidentified metabolitesover 120 minutes. In contrast, human lung microsomes of non-smokers were not active in metabolism of fluensulfone at either 2 or 20 µM fluensulfone incubated for up to 120 minutes (Figure 2). Incubations with a positive control substrate (chlorzoxazone, a probe substrate for CYP 2E1 (human)/cyp 2e1 (mouse)) confirmed that both the mouse and human microsomes had metabolic capacity, although the mouse was more active towards this substrate than human microsomes.

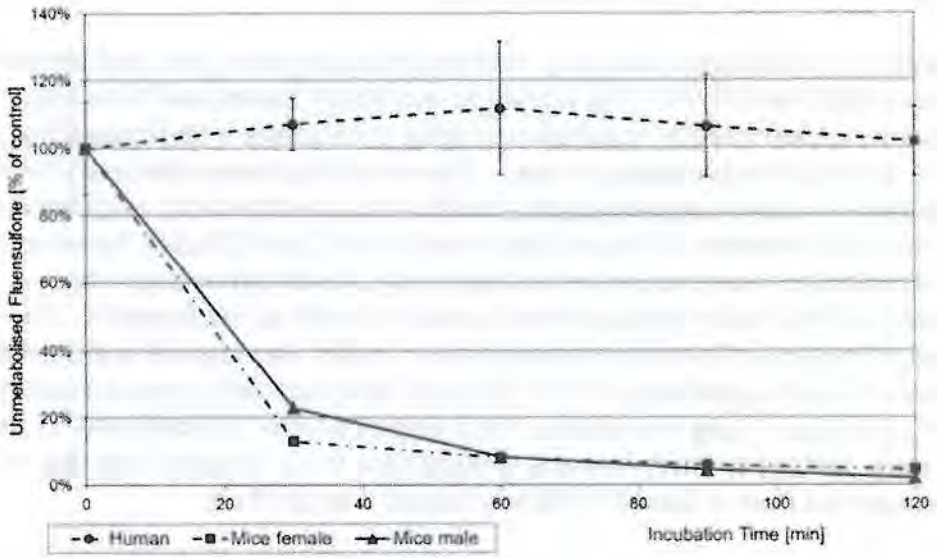

Figure 2. Time-dependent metabolic conversion of fluensulfone in human and mouse lung microsomes.

5-phenyl-1-pentyne, considered a specific inhibitor for CYP 2F enzymes, was used to determine the contribution of mouse cyp 2f2 to the metabolism of fluensulfone in mouse lung microsomes. Incubations with mouse lung microsomes and 2 µM fluensulfone resulted in conversion of approximately 91.3-93.8% of fluensulfone to metabolites as described above (Figure 3, mice female and male with solvent). The addition of 4-methyl pyrazole, an inhibitor of CYP 2E1/cyp 2e1, resulted in a minor increase (~4%) in the amount of unmetabolized fluensulfone indicating that cyp 2e1 likely plays a very minor role in the metabolism of fluensulfone in mouse lung (Figure 3, female and male mice with 4-methyl pyrazole). In the presence of 5 µM 5-phenyl-1-pentyne, 73.1-77% of fluensulfone was converted to metabolites (Figure 3, female and male

mice with 5-phenyl-1-pentyne), indicating that cyp 2f2 is only responsible for ~ 22% of the metabolism of fluensulfone in the mouse lung. The CARC concluded that the data do not support the investigator's conclusion that mouse cyp 2f2 is the predominant enzyme in the metabolism of fluensulfone as postulated in the key events.

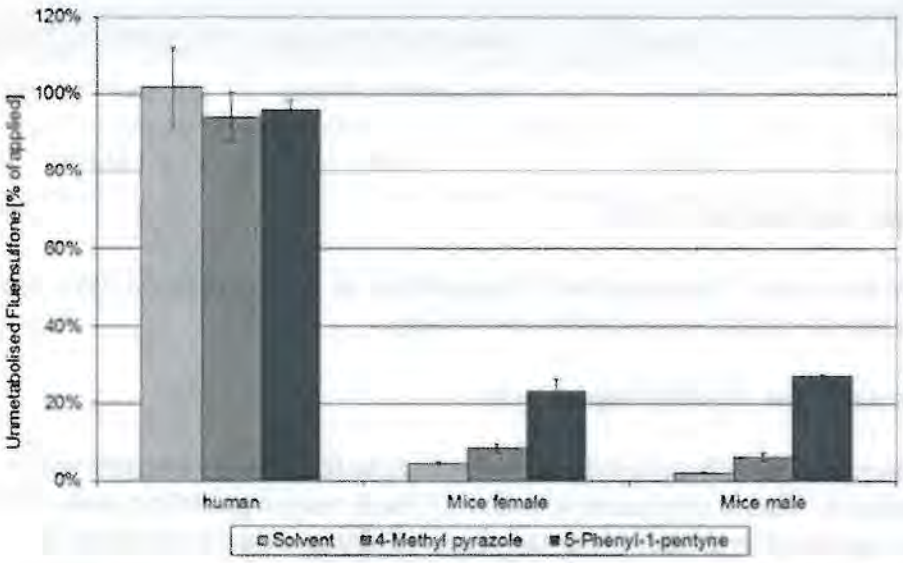

Figure 3. Remaining fluensulfone (%) after 120 minutes incubation in the presence of specific inhibitors for CYP 2E1/cyp 2e1 and cyp 2f2

The data partially supports the key event. Extensive metabolism was observed in mouse but not human lung microsomes; however, the data do not support that cyp 2f2 is the predominant enzyme involved. It must be noted that no data were provided to indicate that metabolism of fluensulfone is a required event in the proposed mode of action. Furthermore, even if a metabolite of fluensulfone is responsible for the subsequent key events, there is no support that metabolism in the lung would be required. The submitted guideline rat metabolism data indicates that fluensulfone is rapidly converted to metabolites and there is uncertainty on how much fluensulfone would reach the lungs to undergo further bioactivation as proposed for the current key event.

2. Key event #2: Terminal bronchiolar cells undergo increased proliferation.

In female CD-1 mice administered 1200 ppm fluensulfone for 3 days, increased cell proliferation was observed as indicated by a significant increase (4.8-fold) in BrdU labeling in the epithelium of the bronchioles in the lung (Table 10). In contrast, mice treated for 7 days did not have increased BrdU labeling. Isoniazid, a known mouse lung tumorigen and positive control for this study, also caused an increase (4.8-fold) in BrdU labeling after 3 days but not 7 days of treatment at 1305 ppm. There was no evidence of necrosis in the lungs of animals treated with fluensulfone or isoniazid. Thus, it is more likely that the proliferative stimulus is a direct mitogenic effect rather than cytotoxicity and regenerative proliferation.

Table 10. BrdU-Labeling index of mouse bronchioles after treatment			
Duration of Exposure	BrdU-positive cells/1000 cells of the bronchiolar epithelium		
	Control	Isoniazid (1305 ppm)	Fluensulfone (1200 ppm)
3 days	25 ± 5	119 ± 69** (4.8-fold)	120 ± 40** (4.8-fold)
7 days	17 ± 10	7 ± 5	13 ± 4

** Fisher's exact test, significant at p <0.01

The data support the key event. Treatment with fluensulfone at 1200 ppm for 3 days, but not 7 days, resulted in increased cellular proliferation in the lung.

3. Key event #3: Bronchiolo-alveolar hyperplasia

No evidence of hyperplasia was observed at either 3 or 7 days following treatment with fluensulfone or isoniazid. This is consistent with the 13-week mouse guideline study (MRID 48574753) where hyperplasia in the lung was not observed using comparable doses of fluensulfone. However, in the 18-month mouse guideline carcinogenicity study (MRID 48574766), bronchiolar hyperplasia was observed in male and female CD-1 mice given 200 or 1200 mg/kg/day in the diet (Table 11). The first observation of hyperplasia was at week 53 in a high dose female that died. It should be noted that the bronchiolar hyperplasia was described as bronchiolization in the original study report.

Table 11. Incidence data of non-neoplastic lesions for male and female CD-1 mice in the 78-week oncogenicity study of fluensulfone.				
	Dose (ppm)			
Males	0	30	200	1200
Bronchiolization (hyperplasia)	1/50	0/50	24*/50	31*/50
Mean severity grade	1.0	-	1.3	1.6
Females				
Bronchiolization (hyperplasia)	5/50	7/50	43*/50	48*/50
Mean severity grade	1.0	1.0	1.8	2.6

* p < 0.01 using Fisher's Exact Test (one-sided)

The data support the key event. Bronchiolo-alveolar hyperplasia was observed in mouse lung following treatment with fluensulfone for 18-months, but was not observed following treatment for 13-weeks.

4. Key event #4: Progression of bronchiolar-alveolar hyperplasia to adenomas and carcinomas

In the guideline carcinogenicity study in mice, an increased incidence of neoplastic lesions was observed in the lungs of females treated with 200 and 1200 ppm of fluensulfone in the diet (Table 12). No statistically significant tumor findings were observed in male mice. As presented in Table 12, increases in the incidence of lung adenomas exceeded the historical control for females at 200 and 1200 ppm. An increase in carcinomas was also observed in female mice at 1200 ppm, although the incidence was not outside the historical control range. The onset of adenomas and carcinomas in the female mice was reduced at 200 and 1200 ppm, when compared to controls and females at 30 ppm, with the earliest onset observed at 1200 ppm. In males there were no differences among control and treated groups in the onset of lung tumors.

Table 12. Fluensulfone – CD-1 Mouse Study (MRID 48574766)
Female Alveolar/Bronchiolar Tumor Rates[+] and Fischer's Exact Test and Exact Trend Test Results

Tumor Type	Dose (ppm)				HC
	0	30	200	1200	
Adenomas (%)	2/50 (4)	4/47 (9)	14/48 (29)	9[a]/49 (18)	9/265 (3%) (0-6%)
p =	0.09379	0.30959	0.00068**	0.02346*	
Carcinomas (%)	2/50 (4)	1/49 (2)	1/49 (2)	4[b]/50 (8)	9/226 (3%) (0-10%)
p =	0.09142	0.87504	0.87504	0.33887	
Combined (%)	3[c]/50 (6)	5/49 (10)	15/49 (31)	12[c]/50 (24)	Not Provided
p =	0.03467*	0.34609	0.00140**	0.01130*	

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52 (adenomas) or week 49 (carcinomas and combined).

[a]First adenoma observed at week 55, dose 1200 ppm.
[b]First carcinoma observed at week 49, dose 1200 ppm.
[c]One animal in each of the control and 1200 ppm dose groups had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

D. Dose-Response and Temporal Association for Key Events and Tumorigenesis

The dose-response and temporal association for key events involved in the development of lung tumors are presented in Table 13. Key event 1 (metabolism by mouse lung) was evaluated *in vitro* and extensive metabolism was observed with 2 µM fluensulfone. Metabolism of fluensulfone in the mouse was not evaluated *in vivo*. Based on the current data it is unclear if metabolism is a required step or that if metabolism is required, it occurs primarily in the lung. Key event 2 (Clara cell proliferation) was an early event observed following 3 days of treatment but absent following 7 days of treatment at1200 ppm, the only dose tested. Since tumors were

observed at 200 ppm, it is not clear if cellular proliferation is occurring at or below doses that induce adenomas. Key event 3 (hyperplasia) occurred after 1 year at 200 ppm and 1200 ppm, along with key event 4 (adenomas/carcinomas).

Table 13. Temporal and dose response for MoA Key Events related to male and female mouse lung tumors

Dose Concordance ↓↓↓↓	Temporal Concordance →→→→							
	Dose (ppm)	Key Event 1	Key Event 2		Key Event 3		Key Event 4	
		Metabolism by mouse lung.	Increased Clara cell proliferation		Bronchiolar-alveolar Hyperplasia (M/F)		Bronchiolar-alveolar Adenoma/Carcinoma (F)	
		In Vitro study only.	3 Days	7 Days	Week 13	Week 53 - 80	Week 13	Week 50 - 80
	0	N/A	--	--	--	--	--	--
	30	N/A	N/A	N/A	N/A	--	N/A	--
	60	N/A	N/A	N/A	--	N/A	--	N/A
	200	N/A	N/A	N/A	N/A	X	N/A	X*
	300	N/A	N/A	N/A	--	N/A	--	N/A
	1200	N/A	X	--	N/A	X	N/A	X
	1500	N/A	N/A	N/A	--	N/A	--	N/A

X = indicate a statistically significant effect compared with concurrent controls. -- indicate no statistically significant effect at the doses tested. n/a = not applicable, doses not tested. * = adenoma only.

E. Strength, Consistency, and Specificity of Tumor Response with Key Events

The strength of the data to support key event 1 is lacking. None of the data presented indicate that metabolic activation is required to elicit any of the subsequent key events. Furthermore, even if metabolic activation to a specific metabolite is required, there is no evidence that this metabolism would occur specifically in the target tissue (lung). Finally, if metabolism in the lung is required, it is clear that cyp 2f2 is not the predominant enzyme involved. The remaining findings in the lungs are consistent between the studies with evidence of early cellular proliferation (3 days, key event 2) and late increases in incidences of hyperplasia (> 13 weeks, key event 3) and adenomas (> 13 weeks, key event 4) at 1200 ppm. The strength of key event 2 is somewhat weak in the regard that there are no data to indicate if increased cellular proliferation was occurring at 200 ppm, a dose where key events 3 and 4 were observed. Key events 3 and 4 were specific in that the only response observed in mice was in the lung and similar effects were not seen in other species. Key event 2 was not tested in other species.

F. Biological Plausibility and Coherence

The postulated MOA for fluensulfone-induction of lung tumors in mice is coherent and plausible. Increased cellular proliferation leading to hyperplasia and subsequent tumors is a common route for non-genotoxic agents. Furthermore, this is the usual sequence of events for lung tumorigenesis in rodents.

G. Alternative Modes of Action for Lung Tumors

Genotoxicity: No evidence for genotoxicity was observed in a battery of genotoxicity tests for fluensulfone and its major metabolites (Tables 7 and 8).

Cytotoxicity: This mode of action is unlikely as no evidence of necrosis or other indicators of cytotoxicity were observed at early time points of administration when increased cellular proliferation was observed.

H. Uncertainties, Inconsistencies, and Database Limitations

There are a few uncertainties with the key events and the data supporting them. The first uncertainty is in key event 1. No data were provided to support that metabolism is a necessary step to result in increased cellular proliferation. Furthermore, even if metabolism is required, there is still an uncertainty on whether the metabolism occurs directly in the lung. The second major uncertainty was that there is a lack of information on cellular proliferation at doses lower than 1200 ppm, despite hyperplasia and adenoma formation occurring at this dose level in the 18-month study. In addition, cell proliferation was only measured in mice; it is unclear if increased cell proliferation is a species specific event. A molecular initiating event preceding the mitogenic burst was not identified for the mode of action and the actual mechanism leading to cellular proliferation is not clearly defined in the registrant's submission (direct mitogenic effect versus cytotoxicity with regenerative proliferation). Finally, there is no clear evidence of Clara cell involvement as the cell origin of proliferation, hyperplasia, or tumors as the cell populations were only identified by eye and not through use of a specific marker.

I. Human Relevance

For the registrants conclusions on the relevance of the MoA to human health see MRID 48575041). The major points of the registrants reasoning are as follows and also illustrated in Table 14:

(i) Mice strains, specifically CD-1 mice, are highly susceptible to induction of lung tumors based on a high spontaneous rate of lung tumors compared to rats and humans, likely because of the unique presence of cyp 2f2 and high number of Clara cells.

(ii) The mechanism of action for fluensulfone is likely similar to isoniazid, another mouse specific lung tumorigen.

(iii) The pathogenesis of lung tumors in mice and humans is different due to distinct differences in lung structure

Table 14. Concordance analysis between mice and humans regarding key events for the mode of action of fluensulfone-induced lung proliferative lesions in mice		
Key Event	**Mice**	**Humans**
Metabolic activation by Cyp 2f2	Yes	No (based on in vitro microsome analysis, known lack of Cyp 2f2 in human Clara cells and fewer Clara cells in humans)
Increased Clara cell proliferation	Yes	Unlikely
Bronchiolo-alveolar hyperplasia and adenoma	Yes	Unlikely

The registrant puts a great deal of weight into the fact that cyp 2f2 activation in the lung is mouse specific. However, there is not enough data to support that: 1) metabolic activation in the lung to an active metabolite is required; 2) if metabolic activation is necessary, activation in the target tissue is required; and 3) cyp 2f2 is the predominant enzyme involved in metabolism of fluensulfone in the mouse lung. In particular, the data showed that cyp 2f2 in mouse lung is only responsible for, at most 20.7% of the metabolism that occurs in the lung, and therefore cyp 2f2 is likely not the major enzyme involved.

Furthermore, while humans have a relatively lower number of Clara cells compared to mice, Clara cells are still present in humans. No data were provided by the registrant to support that cell proliferation does not occur in other species such as the rat or clear evidence that the cellular proliferation was limited to Clara cells. Thus, the postulated mode of action could still occur in humans leading to increased proliferation of Clara cells or other cell types.

Taken together, there are still a number of uncertainties in the data to exclude the human relevance of the current proposed MOA.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE EVIDENCE

Fluensulfone was administered in the diet to Wistar rats (50/sex/dose) at dose levels of 0/0, 1.4/1.7, 9.6/11.6, and 57.7/69.3 mg/kg bw/day, for 104 weeks. Fluensulfone was administered in the diet to CD-1 mice (50/sex/dose) at dose levels of 0/0, 4.2/6.4, 27.6/39.0, and 152.3/188.4 mg/kg bw/day, for 78 weeks.

The CARC considered the following for a weight-of-evidence determination of the carcinogenic potential of fluensulfone.

Evidence for Carcinogenicity

Rat

No tumors were observed.

Adequacy of Dosing: The doses tested were considered to be adequate, but not excessive, in both sexes to assess the carcinogenic potential of fluensulfone. This determination was based on the results of the 90-day study which used for dose selection and the presence of non-neoplastic lesions in the main study.

Mouse

Lung tumors: Female mice had a significant trend for alveolar/bronchiolar combined adenomas and/or carcinomas at $p < 0.05$. There were significant pair-wise comparisons of the 200 ppm dose group with the controls for alveolar/bronchiolar adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. There were also significant pair-wise comparisons of the 1200 ppm dose group with the controls for alveolar/bronchiolar adenomas and combined adenomas and/or carcinomas, both at $p < 0.05$. No statistically significant tumor findings were observed in male mice. These tumors were corroborated by the presence of non-neoplastic lesions (bronchiolization, a type of hyperplasia) in both male and female mice. **The CARC considered the lung tumors in female mice to be treatment-related.**

Adequacy of Dosing: Dosing was considered adequate and not excessive. This determination was based on the results of the 90-day study which was used for dose selection and the presence of non-neoplastic (both sexes) and neoplastic lesions (females only) in mice in the main study.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005): **Fluensulfone is classified as "Suggestive Evidence of Carcinogenic Potential"** based on the following considerations:

(i) Treatment-related alveolar/bronchiolar adenomas of the lung in female mice at doses that were considered adequate to assess carcinogenicity;

(ii) No treatment-related tumors were observed in either sex of rats at doses that were considered adequate to assess carcinogenicity;

(iii) There is no mutagenicity concern from the *in vivo* or *in vitro* genetic toxicity assays; and

(iv) There is insufficient data to support the proposed non-genotoxic MOA.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC recommended using a non-linear approach (i.e., reference dose (RfD)) that will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to fluensulfone.

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